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**Primary Eye Irritation
of
Guanidine Hydrochloride in Rabbits**

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Thomas P. Kellner, BA, SP5
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**MAMMALIAN TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY**

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Primary Eye Irritation of Guanidine Hydrochloride in Rabbits
(Toxicology Series 97)--Brown et al

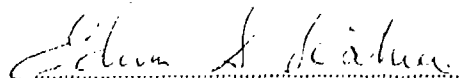
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In conducting the research described in this report, the investigation adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care, Institute of Laboratory Animal Resources, National Research Council.

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ABSTRACT

The primary eye irritation potential of guanidine hydrochloride was determined in male New Zealand White rabbits using a modified Draize method. The compound produced irritation in all animals tested. Signs of irritation were erythema and chemosis of the conjunctiva, iritis, and corneal lesions. These results indicate that guanidine hydrochloride should be classified as an ocular irritant.

Key words: Primary Eye Irritation, Guanidine Hydrochloride, Mammalian Toxicology, Nitroguanidine, Munitions, Explosives, Rabbit

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PREFACE

TYPE REPORT: Primary Eye Irritation GLP Study Report

TESTING FACILITY: US Army Medical Research and Development
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Presidio of San Francisco, CA 94129-6800

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US Army Biomedical Research and Development
Laboratory
Fort Detrick, Maryland 21701-5010
Project Officer: Gunda Reddy, PhD

WORK UNIT: 3E162720A835; WU 180; APC TL09

GLP STUDY NUMBER: 84004

STUDY DIRECTOR: MAJ Don W. Korte Jr, PhD, MSC

PRINCIPAL INVESTIGATOR: MAJ Larry D. Brown, DVM, MPVM, VC
Diplomate of American College of
Veterinary Preventive Medicine

CO-PRINCIPAL INVESTIGATOR: Thomas P. Kellner, BA, SP5

REPORT AND DATA MANAGEMENT: A copy of the final report,
study protocol, raw data,
analytical, stability, and
purity data of the test
compound, and an aliquot of
the test compound will be
retained in the LAIR Archives.

TEST SUBSTANCE: Guanidine Hydrochloride

INCLUSIVE STUDY DATES: 3 May - 19 Jun 84

OBJECTIVE: The objective of this study was to determine
the primary eye irritation potential of
guanidine hydrochloride in male New Zealand
White rabbits.

ACKNOWLEDGMENT

The authors wish to thank the following individuals for their contributions to the successful completion of this study: SP4 Paul D. Mauk, BS, SP4 Steven K. Sano, BS, Gerald F.G. Hiatt, PhD, and Yvonne C. Johnson, BS, for research assistance; Richard J. O'Connor, MS, for chemical analysis; Richard D. Spieler, Richard Katona, Roosevelt Cunningham, Charlotte Speckman, and Edward M. Sands for animal care and facility management, and Callie Crosby, MA, JoAnn Nishimoto, and Dianna B. Johnson for secretarial assistance.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED
IN THE STUDY

We, the undersigned, declare that GLP Study 84004 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

Don W. Korte / 16 June 87
DON W. KORTE JR., PhD / DATE
MAJ, MSC
Study Director

Larry D. Brown / 17 June 87
LARRY D. BROWN, DVM / DATE
MAJ, VC
Principal Investigator

Thomas P. Kellner / 14 Jul 87
THOMAS P. KELLNER, BA / DATE
SP5, USA
Co-Principal Investigator

Conrad Wheeler / 18 June 87
CONRAD WHEELER, PhD / DATE
DAC Analytical Chemist



DEPARTMENT OF THE ARMY
LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA

14 July 1987

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

1. I hereby certify that in relation to LAIR GLP Study 84004 the following inspections were made:

21 May 1984 - Predosing Examination

19 June 1984 - 14-Day Observation

2. The report and raw data for this study were audited on 23 March 1987.

Carolyn M. Lewis
CAROLYN M. LEWIS
DAC
C, Quality Assurance Section

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Primary Eye Irritation of Guanidine Hydrochloride in Male Rabbits -- Brown et al

Nitroguanidine, a primary component of US Army triple-base propellants, is now produced in a Government-owned contractor-operated ammunition plant. The US Army Biomedical Research and Development Laboratory (USABRDL), as part of its mission to evaluate the environmental and health hazards of military-unique pollutants generated by US Army munitions manufacturing facilities, conducted a review of the nitroguanidine data base and identified significant gaps in the toxicity data (1). The Division of Toxicology, LAIR, was tasked by USABRDL to develop a genetic and mammalian toxicity profile for nitroguanidine, related intermediates/by-products of its manufacture, and its environmental degradation products.

Objective of Study

The objective of this study was to determine the primary eye irritation potential of guanidine hydrochloride in male New Zealand White rabbits.

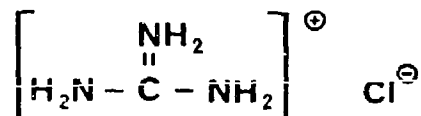
MATERIALS

Test Substance

Chemical name: Guanidine Hydrochloride

Chemical Abstract Service Registry No.: 050-01-1

Chemical structure:



Molecular formula: CH_6ClN_3

Other test substance information is presented in Appendix A.

Vehicle

None

Animal Data

Six male New Zealand White rabbits (Elkhorn Rabbitry, 5265 Starr Way, Watsonville, CA) were identified individually with ear tattoos numbered 84F341, 84F342, 84F343, 84F344, 84F345, and 84F346. The animal weights on dosing day (22 May and 5 Jun) ranged from 2.3 to 2.9 kg. Additional animal data appear in Appendix B.

Husbandry

The rabbits were housed individually in stainless steel, screen-bottomed, battery-type cages with automatic flushing dumptanks. The diet consisted of 150 g/day of Certified Purina Chow Diet 5322 (Ralston Purina Company, St. Louis, MO); water was provided by continuous drip from a central line. The animal room temperature was maintained at 16.1 to 20.0°C except for a spike to 21.1°C during a steam outage. The relative humidity ranged from 40 to 70 percent. The photoperiod was 12 hours of light per day.

METHODS

This study was conducted in accordance with guidelines promulgated by the EPA for ocular irritation testing (2) and LAIR SOP-OP-STX-33 (3).

Group Assignment/Acclimation

After 14 days of quarantine by the LAIR Animal Resources Group, the six study rabbits were transferred to the Toxicology Suite. Animals were divided into two dose groups of 3 males each and were acclimated to the Toxicology Suite for 5 days before the day of dosing. During this period they were observed daily for signs of illness.

Dosage Levels

One-tenth milliliter of guanidine hydrochloride was administered once to the right eye of each rabbit by gently pulling the lower lid away from the conjunctival cul-de-sac to form a cup into which the compound was dropped. The upper and lower lids were then held together for one second to prevent loss of material. The first group, (A), was dosed on 22 May, and the second group, (B), was dosed on 5 June.

Compound Preparation

Since the dose of guanidine hydrochloride administered was expressed in terms of milliliters, it was necessary to equate milliliters to grams because the test compound was administered in crystalline powder form. By using a pipette, it was determined after five measurements that 0.1 ml of guanidine hydrochloride tightly packed weighed a mean average of $81.4 \text{ mg} \pm 1.0 \text{ mg}$ (SE).

Test Procedures

On 21 May, both eyes of Group A animals were examined, unaided and with .2% fluorescein dye solution, for any corneal, iridial, or conjunctival abnormalities. On 22 May, 0.1 ml of guanidine hydrochloride was placed in the right eye of each rabbit. The left eye was untreated and served as the control. Group B rabbits were examined on 4 June and dosed on 5 June.

Observations

The grading and scoring for ocular reactions were performed according to Table 1 (3). Observations were made daily from 22 May to 15 June 1984 for Group A and 5 June to 19 June 1984 for Group B. Scoring and grading of ocular reactions were performed at 1 hour, 1, 2, 3, 4, 7, and 14 days for both groups A and B. Fluorescein dye was used for scoring and grading at 24 hours, 7, and 14 days.

TABLE 1
GRADES FOR OCULAR LESIONS

CORNEA

Opacity: degree of density (area of greatest density taken for reading) No ulceration or opacity.....	0
Scattered or diffuse areas of opacity (other than slight dulling of normal luster) details of iris clearly visible.....	1*
Easily discernible translucent areas, details of iris slightly obscured.....	2
Nacreous areas, no details of iris visible, size of pupil barely discernible.....	3
Opaque cornea, iris not discernible through opacity.....	4

IRIS

Normal.....	0
Markedly deepened rugae, congestion, swelling, moderate circumferential hyperemia or injection, any of these or any combination thereof, iris still reacting to light (sluggish reaction is positive).....	1*
No reaction to light, hemorrhage, gross destruction (any or all of these).....	2

CONJUNCTIVA

Redness (refers to palpebral and bulbar conjunctiva, excluding cornea and iris)	
Blood vessels normal.....	0
Some blood vessels definitely hyperemic (injected).....	1
Diffuse, crimson color, individual vessels not easily discernible....	2*
Diffuse beefy red.....	3
Chemosis: lids and/or nictitating membranes	
No swelling.....	0
Any swelling above normal (including nictitating membranes).....	1
Obvious swelling with partial eversion of lids.....	2*
Swelling with lids about half-closed.....	3
Swelling with lids more than half-closed.....	4
*Indicates minimum level for a positive response	

Duration of Study

Appendix C is a complete listing of historical events.

Changes/Deviations from Original Protocol

Animals were terminated by intravenous overdose of T-61® Euthanasia Solution (American Hoechst Corp., Somerville, NJ) and not sodium pentobarbital as described in the protocol. The diet consisted of 150 g of rabbit chow daily and not 110 g as described in the protocol. Animals were not weighed on 25 May 1984 as planned. These deviations did not impact upon the outcome of this study.

Raw Data and Final Report Storage

A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

RESULTS

Results from scoring the ocular irritation in each rabbit are tabulated in Appendix D.

Slight corneal opacity (score 1) was observed in three of six rabbits tested. The opacity in rabbit 84F342 was first observed at 1-hour, persisted through the 4-day observation, and cleared by the 7-day observation. The opacities in rabbits 84F343 and 84F344 first appeared at 48 hours and had disappeared at 7 days and 72 hours, respectively. All six rabbits had normal appearing corneas by unaided examination on days 7 and 14.

In two (84F342 and 84F343) of six rabbits, slight (score 1) iritis (vascular injection) was observed. This was first noted on the 24-hour observation and had cleared in both animals by day 7. A score of one is considered positive for iritis.

In evaluating the conjunctiva for both redness and chemosis, five of six rabbits showed a positive reaction at some point after dosing. All rabbits exhibited slight conjunctival redness (score 1). This condition was present in 5 of 6 at the 1-hour observation. The redness in all animals had cleared by the 14-day observation except for animal 84F346, which had slight redness remaining under the upper eyelid. Rabbits 84F341, 84F342, 84F344, and 84F346 showed moderate redness (score 2) on the 48-hour observation period. Conjunctival chemosis (score 1 or 2)

was observed in all rabbits by the 1-hour observation. The swelling had cleared by day 14. Four of six rabbits showed moderate (score 2) conjunctival chemosis.

In addition to the ocular reactions described in the above paragraphs, one rabbit (84F343) developed corneal erosions, which resolved into a small 4-5 mm erosion which was visible only with fluorescein staining on day 14. Also, a large area of the nictitating membrane became devitalized in five rabbits. This tissue was sloughed in some but not all animals. The membrane had returned to normal by 14 days. Other ocular reactions observed were squinting, slight to marked tearing, and free-floating exudate. The control (left) eye in each animal remained normal throughout the study.

DISCUSSION

The EPA recommendations follow the Consumer Product Safety Commission guidelines on the scoring of ocular irritation tests (2). These guidelines state that an animal is considered to have exhibited a positive reaction if the test substance produces one or more of the following signs: ulceration of the cornea (other than a fine stippling); opacity of the cornea (other than a slight dulling of the normal luster); inflammation of the iris (other than a slight deepening of the rugae or a slight hyperemia of the circumcorneal blood vessels); an obvious swelling in the conjunctiva (excluding the cornea and iris) with partial eversion of the lids; or a diffuse crimson-red coloration in the conjunctiva with individual vessels not easily discernible. The test shall be considered positive if four or more of the animals in the test group exhibit a positive reaction. The EPA also classifies irritancy in terms of duration of response. A test compound which produces a response which is reversible in 21 days is classified as irritative while a test compound that produces an irreversible response (present at 21 days) is classified as corrosive. This system requires that classification be dependent on the most severe responder. Thus, according to EPA guidelines, since five of six animals exhibited a score of 2 (positive reaction) for either conjunctival redness or conjunctival chemosis, the primary ocular irritation test for guanidine hydrochloride was positive in male New Zealand White rabbits. All positive responses had resolved by day 14. The corneal erosion observed on day 14 in rabbit 84F343 could only be detected by the fluorescein dye procedure and was beyond the threshold for visible grading. The conjunctival redness observed in rabbit 84F346 at day 14 was graded as slight.

Since this represented resolution of the initial lesion and the threshold score for a positive response is 2 (moderate), this lesion was classified as reversible. Based on these findings the study was terminated at day 14 and the compound was classified as an irritant.

The corneal erosions observed may be caused in part by the physical state of the compound, and in part because guanidine hydrochloride was not irrigated from the eye. If the eye had been irrigated with large volumes of water, the compound would have been washed from the eye. Thus, prompt and thorough irrigation of the eye may ameliorate, if not completely eliminate, any clinical symptomatology or ocular damage.

CONCLUSION

Guanidine hydrochloride is an ocular irritant in male New Zealand White rabbits because it produced a positive test response which was reversed in 14 days.

REFERENCES

1. Holleman JW, Ross RH, Carroll JW. Problem definition study on the health effects of diethyleneglycol dinitrate, triethyleneglycol dinitrate, and trimethylolethane trinitrate and their respective combustion products. Frederick, Maryland: US Army Biomedical Research and Development Laboratory, 1983, DTIC No. ADA 127846.
2. Environmental Protection Agency. Office of Pesticides and Toxic Substances, Office of Toxic Substances (TS-792). Primary Eye Irritation. In: Health effects test guidelines. Washington, DC: Environmental Protection Agency, August 1982; EPA 560/6-82-001.
3. Primary Eye Irritation Study. LAIR Standard Operating Procedure OP-STX-33, Letterman Army Institute of Research, Presidio of San Francisco, CA. 5 March 1982.

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APPENDICES

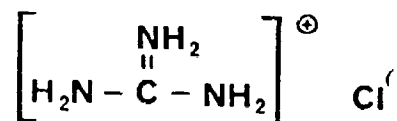
CHEMICAL DATA

Chemical name: Guanidine Hydrochloride

Alternate chemical name: Aminomethanamiđine hydrochloride,
Carbamamidine hydrochloride,
Carbamidine hydrochloride,
Aminoformamidine hydrochloride,
Iminourea hydrochloride

Chemical Abstracts Service Registry No.: 50-01-1

Chemical structure:



Molecular formula: CH_6ClN_3

Molecular weight: 95.5

Physical state: White powder

Melting point: 182-184°C (184-185°C*)

Analytical data/purity: Water content 0.1% by Karl Fischer analysis.* The material is at least 98% pure and chromatographs as one spot by thin layer chromatography.† Elemental analysis. Calculated for CH_6ClN_3 , Cl, 37.1. Found: Cl, 36.6.† An IR spectrum was obtained upon receipt of the compound. IR(KBr): 3400, 2750, 1650, 1535, 1050 (broad) cm^{-1} . A comparison of this spectrum to the Sadtler standard spectrum confirmed the identity of the material.‡

Source: Sigma Chemical Co.
St. Louis, MO

Lot number: 103F-5623

*Zygmunt R., Analytical data sheet for guanidine hydrochloride, lot number 103F-5623. Sigma Chemical Co., St. Louis, 16 Feb 84.

†Sigma Chemical Company, St. Louis, MO. Becky Goodloe, PhD, personal communication, 5 March 1985.

‡Sadtler Research Laboratory, Inc., Sadtler standard spectra, Philadelphia: The Sadtler Research Laboratory, Inc., 1962: Infrared Spectrogram #8676.

Stability in vehicle: A preliminary study was conducted to determine the stability of guanidine hydrochloride in the vehicle, sterile water for injection. A solution of guanidine hydrochloride (18.825 ug/ml water) was assayed after preparation and 4 hours later by using the Voges-Proskauer Method (Micklus MJ, Stein IM. The colorimetric determination of mono- and disubstituted guanidines. Anal Biochem 1973;54:545-533). This method is specific for unsubstituted and monosubstituted guanidines and yields a colored derivative which is monitored spectrophotometrically. Three samples were analyzed for each time point and the results were as follows.

Absorbance Value (1st Assay)	Absorbance Value (2nd Assay)
2.190	2.053
2.165	2.190
2.160	2.191
$\bar{x} = 2.172$	$\bar{x} = 2.145$

The values for the two assays were within 1.5 percent of each other which is within the error for repeated sampling using this test. This indicates that the compound is stable in aqueous solution for at least 4 hours.

APPENDIX A (concluded)

ANIMAL DATA

Species: Oryctolagus cuniculus

Strain: New Zealand White (albino)

Source: Elkhorn Rabbitry
5265 Starr Way
Watsonville, CA 95076

Sex: Male

Age: Young Adults

Animals in each group: 3 males

Condition of animals at start of study: Normal

Body weight range at dosing: 2.3 - 2.9 kg

Identification procedures: Ear tattoo; numbers 84F341,
84F342, 84F343, 84F344, 84F345,
84F346.

Pretest conditioning:

1. Quarantine from 3 May to 17 May 1984
2. Animal eyes were examined 24 hours before dosing using .2% fluorescein dye solution and ultraviolet light (long wave).

Justification: Laboratory rabbits are a proven sensitive animal model for ocular testing.

HISTORICAL LISTING OF STUDY EVENTS

<u>Date</u>	<u>Event</u>
3 May 84	Animals received at LAIR, tattooed, examined for illness and placed under quarantine by the Animal Resources Group.
17 May 84	Animals transferred to Toxicology Suite, separated into test groups and weighed.
21 May 84	Group A rabbits (84F341,342,343) examined for pre-existing ocular injury.
22 May 84	Group A rabbits weighed and dosed. One hour post-exposure scores were performed. Group B rabbits weighed.
22 May-5 Jun 84	Group A rabbits were observed daily.
23 May 84	Group A 24-hour post-exposure scores were performed.
24 May 84	Group A 48-hour post-exposure scores were performed.
25 May 84	Group A 72-hour post-exposure scores were performed.
26 May 84	Group A 4-day post-exposure scores were performed.
29 May 84	Group A 7-day post-exposure scores were performed.
1 June 84	All animals weighed.
4 Jun 84	Group B rabbits (84F344,345,346) examined for pre-existing ocular injury.

APPENDIX C

5 Jun 84	Group A 14-day post-exposure scores were performed, animals weighed and sacrificed.
5 Jun 84	Group B rabbits weighed and dosed. One hour post-exposure scores were performed.
6 Jun 84	Group B 24-hour post-exposure scores were performed.
7 Jun 84	Group B 48-hour post-exposure scores were performed.
8 Jun 84	Group B 72-hour post-exposure scores were performed. Animals were weighed.
9 Jun 84	4-day post-exposure scores were performed.
10-19 Jun 84	Group B rabbits were observed daily.
12 Jun 84	Group B 7-day post-exposure scores were performed.
15 June 84	Animals were weighed.
19 Jun 84	Group B animals were weighed and 14-day post-exposure scores were performed. Animals were sacrificed and the study terminated.

TABULAR SCORING DATA

on

ACUTE EYE IRRITATION SUMMARY FORMS

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APPENDIX D

FIGURE 1

SUMMARY OF CORNEA SCORES

Rabbit No.	base- line	Score by Animal						
		1 hr	24 hr	48 hr	72 hr	4 d	7 d	14 d
84F341	0*	0	0	0	0	0	0	0
84F342	0	1	1	1	1	1	0	0
84F343	0	0	0	1	1	1	0	0
84F344	0	0	0	1	0	0	0	0
84F345	0	0	0	0	0	0	0	0
84F346	0	0	0	0	0	0	0	0

* For scoring see Table I

FIGURE 2
SUMMARY OF IRIS SCORES

Rabbit No.	Score by Animal							
	base- line	1 hr	24 hr	48 hr	72 hr	4d	7 d	14 d
84F341	0	0	0	0	0	0	0	0
84F342	0	0	1	1	1	1	0	0
84F343	0	0	1	0	0	0	0	0
84F344	0	0	0	0	0	0	0	0
84F345	0	0	0	0	0	0	0	0
84F346	0	0	0	0	0	0	0	0

FIGURE 3
SUMMARY OF CONJUNCTIVAL REDNESS SCORES

Rabbit No.	base- line	Score of Animal						
		1 hr	24 hr	48 hr	72 hr	4 d	7 d	14 d
84F341	0	1	1	2	1	1	1	0
84F342	0	1	2	2	2	2	1	0
84F343	0	0	1	1	1	0	1	0
84F344	0	1	1	2	2	2	1	0
84F345	0	1	1	1	1	1	0	0
84F346	0	1	1	2	2	2	1	1

FIGURE 4
SUMMARY OF CONJUNCTIVAL CHEMOSIS SCORES

Rabbit No.	Score by Animal							
	base- line	1 hr	24 hr	48 hr	72 hr	4 d	7 d	14 d
84F341	0	1	0	0	0	0	0	0
84F342	0	2	1	1	1	2	1	0
84F343	0	1	2	1	0	1	0	0
84F344	0	2	2	2	1	1	1	0
84F345	0	1	1	1	0	0	0	0
84F346	0	2	1	2	1	1	1	0

Figure 5

Clinical Description of Ocular Lesions by Animal
(Supplemental to Clinical Signs Reported on Figures 1-4)

Animal 84F341

Slight injection, margin 3rd eyelid - 1 hr
Moderate tearing - 48 hrs
Exudate in lower cul-de-sac (necrotic tissue) - 48
hrs, 72 hrs
Slight tearing - 72 hrs
Third eyelid covered with exudate (sloughing
epithelium) - 72 hrs
Exudate on 3rd eyelid and cornea - 4 days
Marked squinting, right eye - 1 hr
Moderate protein exudate - 24 hrs

Animal 84F342

Slight squint - 24 hrs
Slight tearing - 24 hrs, 72 hrs
Corneal opacity/clouding - 24 hrs, 48 hrs
Blanching of nictitating membrane/sloughing
epithelium - 24 hrs
Lids red and swollen - 24 hrs, 48 hrs, 72 hrs
Moderate tearing - 1 hr, 48 hrs
Diptheritic membrane in lower cul-de-sac - 72 hrs
Eversion of lid - 4 days
Protein exudate on conjunctiva - 1 hr
Palpebrae swelling - 1 hr
Moderate squinting, right eye - 1 hr

Animal 84F343

Slough of conjunctival membrane at fornix - 24 hrs
Moderate protein exudate - 24 hrs
Slight tearing - 24 hrs
Moderate tearing - 48 hrs
Diffuse area on cornea - 48 hr
Necrotic conjunctival epithelium in lower cul-de-sac - 48 hrs
Diffuse area of corneal erosion - 72 hrs
Diphtheritic membrane lower cul-de-sac - 72 hrs
Third eyelid edematous - 1 hr
Fluorescent dye staining area, cornea - 14 days
Slight squint - 1 hr
Moderate tearing - 1 hr
Slight exudate - 1 hr

Animal 84F344

Marked tearing - 1 hr
Palpebrae red slight - 24 hrs
White avascular necrotic epithelium, 3rd eyelid and lower fossa - 24 hrs, 48 hrs, 72 hrs, 4 days
Yellow discharge on lower lid (hairs) - 24 hrs
Ocular discharge - 48 hrs
Lids swollen markedly - 48 hrs
Sloughing diphtheritic membrane, 3rd eyelid - 7 days

Animal 84F345

Slight tearing - 1 hr
Third eyelid vessels injected - 24 hrs, 48 hrs, 72 hrs, 4 days
White protein exudate conjunctiva - 24 hrs
Yellow stained hairs, lower lid - 24 hrs

Animal 84F346

Slight tearing - 1 hr, 24 hrs
Yellow-Brown exudate over 3rd eyelid - 1 hr
White necrotic avascular epithelium over 3rd eyelid - 24 hrs, 48 hrs, 72 hrs
White necrotic avascular epithelium lower conjunctiva - 24 hrs
White patches on third eyelid - 4 days
Sloughing diphtheritic membrane over 3rd eyelid - 7 days
Palpebrae red slight - 24 hrs

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